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Short communication

Phytochemical profiling and antioxidant potential of methanolic extract of *Vitis vinifera L* seeds

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Abstract

Background: Grapes are the most widely consumed fruit worldwide. Grape seeds are a natural source of polyphenols that possess pharmacological and therapeutic activities. The grape seeds have long been used in traditional medicine to alleviate various illnesses.
Objective: This study aimed to identify the major phytocompounds of grape seed methanolic extract and evaluate its antioxidant activity.
Methods: The methanolic extract of grape seeds was subjected to gas chromatography–mass spectrometry to identify the major phytochemical compounds, and further antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as per the guidelines of the Association of Official Analytical Chemist.
Results: In total, 10 phytochemical compounds (1, 3, 5-benzenetriol, 1, 3-benzenediol, benzoic acid, reticuline, 2-hydroxy-5-methylisophthalaldehyde, 4-vinylbenzene-1, 2-diol, cyclohexane carboxylic acid, eicosanoic acid, octadecanoic acid, and 2, 6, 10, 14, 18-pentamethy l - 2, 6, 10, 14, 18-eicosapentaene) were identified in the methanol extract. By the DPPH method, the antioxidant activity of the extract was 212.6 µg/kg.
Conclusion: Phytochemical profiling of the methanolic extract of grape seeds revealed the presence of phenols (1,3-benzenediol, 4-vinylbenzene-1,2-diol), polyphenol (1,3,5-benzenetriol), phenolic acid (benzoic acid), alkaloid (reticuline), aldehyde (2-hydroxy-5-methylisophthalaldehyde), straight-chain saturated fatty acid (eicosanoic acid and octadecanoic acid), and carboxylic acid (cyclohexane carboxylic acid). Of these phytocompounds, benzoic acid and reticuline are potent natural antioxidants that would be effective against free radicals/reactive oxygen species with wider therapeutic applications in metabolic disorders, inflammatory conditions, and cancer.

Keywords: Antioxidants, gas chromatography–mass spectrometry, grape seed extract, polyphenols, phytocompounds.

Grape seeds (*Vitis vinifera L.*) are generally considered a nonedible part of the grapefruit and are a waste or by-product of the wine industry. Grape seeds constitute approximately 3.0% of the weight of the fruit and are a rich source of polyphenolic compounds. [1] Owing to their abundant phenolic content, they may serve as an affordable source of antioxidant and antimicrobial agents. The chemical composition of grape seed extracts includes complex carbohydrates (29.2%), fibers (40.0%), oil (16.0%), proteins (11.0%), and polyphenols (7.0%) as well as vitamins, sugars, mineral salts, and tannins. [2, 3] However, the phenolic constituents of the grapes may vary according to the species, cultivational techniques, and environmental conditions.[4]

Phytocompounds have long been explored as potent antioxidants that can scavenge reactive oxygen species (ROS). Prolonged inflammation in chronic microbial infections is often associated with consistent upregulation of proinflammatory signals that lead to

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the release of lysosomal enzymes and free radicals, such as ROS, a characteristic feature of chronic inflammation. ROS overproduction and diminished antioxidant activity are the hallmarks of metabolic syndrome. ROS overproduction leads to oxidative stress and takes on a critical role in both metabolic and pathological disorders, namely, diabetes, obesity, dyslipidemia, insulin resistance, chronic inflammation, and cardiovascular diseases. [5 - 7] Excess ROS that results in oxidative stress is proven to induce the oxidation of biological substances, cellular damage, DNA damage, and peroxidize lipids. [8, 9] Antioxidants are free radical scavengers that play a major role in minimizing oxidative damage in the cells, cellular constituents, and tissues thereby curtailing the aging processes and stress-associated illnesses in humans. [10]

Various studies have validated the significance of grape seed extracts in food, cosmetic, and pharmaceutical industries. In medicine, grape seed extracts act as a powerful antioxidant to secure the body from disease, decay, and premature aging by oxygen-free radical scavenging effect. It acts as an anti-inflammatory substance that catalyzes the enzyme to release histamine in inflammation and allergies. Owing to their broad-spectrum antimicrobial activity and extraordinary pharmacological properties, plant phenolic compounds are considered remarkable natural bioactive chemicals. Based on the number of aromatic rings present and linking structural elements, phenolic compounds are categorized as flavonoids and non-flavonoids. [11, 12] Plants synthesize both enzymatic and non-enzymatic antioxidants in response to oxidative stress: biotic and abiotic stress. Among the non-enzymatic antioxidants, flavonoids, alkaloids, and phenolic compounds are documented to protect them from oxidative damage. These compounds are potential antioxidants that can be beneficial to human health. [12]

Grape seed extracts contain major amounts of linoleic fatty acid, which reduces total blood cholesterol and low-density lipoprotein cholesterol, thereby reducing the formation of arteriosclerosis, cardiovascular diseases, autoimmune disorders, and cancer. Grape seed extract has antihypertensive activity by suppressing oxidative stress and vasodilation (by inhibiting angiotensin-converting enzyme and nitric oxide). [3, 13, 14]

Grape seeds possess phytopharmaceutical compounds, namely, alkaloids, flavonoids (quercetin, prosiadine, catechins, resveratrol, and anthocyanins), and polyphenols (mixture of proanthocyanidins, monomers, and oligomers). Grape seed polyphenols have a superior antioxidant properties and reduce the risks of chronic illness. Grape seed extract contains proanthocyanidin that inhibits lipid peroxidation and causes oxidative injury in the intestinal membrane by microviscosity in the gastric and duodenal membranes. Resveratrol (polyphenol) in grape seed extract exhibits an antibacterial activity, which suppresses the growth of *Helicobacter pylori*, and has ulcer-healing activity by the stimulation of COX1, which leads to improved angiogenesis. [13, 15]

This study aimed to identify the bioactive compounds present in the methanolic extract of grape seeds by gas chromatography–mass spectrometry (GC–MS) and evaluate the antioxidant activity by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.

Materials and methods

GC–MS analysis of the methanolic extract of grape seeds

The study protocol was reviewed and approved by the institutional review board (SBDCH–IRB-PG/ 22-11/09) of Sree Balaji Dental College and Hospital, BIHER, Chennai, India. The GC–MS analysis of the methanolic extract of grape seeds was performed in GC–MS QP2010 Plus, GC 6890 model series (Shimadzu, Japan) equipped with a flame ionization detector. The chromatographic capillary column of the GC equipped with SH-I-624Sil MS measured 30 m in length and 0.5 mm in diameter. The film thickness of the column was 1.5 μm coated with the silarylene phase (6.0% cyanopropylphenyl/ 94.0% dimethyl polysiloxane) and connected with a flame ionization detector (FID). The sample was prepared in the concentration of 10 mg in 50 mL of methanol. The initial (hold) temperature of the GC was at 70°C for 30 min. The injection volume was 1 mL, which was injected with a split ratio of 30:1. Helium carrier gas was used at a flow rate of 1 mL/min. The total run time was 24 min, and the chromatographic column finally reached a temperature of 200°C maintained for 0.5 min at the end of the process. After the compounds in the column had separated, the peaks (mass spectra) of the compounds were obtained at 70 eV by electron ionization and further analyzed by an FID detector. To determine the structure, molecular weight, and names of the compounds, the spectrum of the compounds in the sample was identified by comparing it with the spectrum of known compounds in the NIST 20 M1 structural library. [16 - 18]

Antioxidant activity of the methanolic extract of grape seeds

The antioxidant activity of the methanolic extract of grape seeds was evaluated as per the Association of Official Analytical Chemist guidelines 2012.04 by the DPPH method. [19] For the DPPH reagent preparation, 7.9 mg of DPPH was mixed with 100 mL of ethanol (99.5%) and kept undisturbed for 2 h in the dark at room temperature. The grape seed extract (1 mL) was mixed with the DPPH solution (1 mL) and used as the test. Methanol (1 mL) in the DPPH solution (1 mL) was used as blank. The test sample and blank solution were vigorously shaken and kept undisturbed for 30 min at room temperature in pitch dark. After incubation, the absorbance for the test and blank was measured at 515 nm using DLAB SP-UV1000 SPECTROPHOTOMETER (CHINA).

The antioxidant activity was measured by the formula, % Radical scavenging activity = (A1–A2) × 100/A1, where A1 = absorbance of blank and A2 = absorbance of sample solution. The DPPH free radical scavenging activity and inhibitory concentration$_{50}$ (IC_{50}) were further calculated using above values. [20 - 22]

Results

Analysis of the spectrum of the GC–MS chromatogram of the methanolic extract of grape seeds showed 10 peaks **(Figure 1)**. The peaks of the bioactive compounds were identified as major phytocompounds.

According to the GC–MS QP2010 Plus (Shimadzu), the percentage peak method was used to identify the quantity of the bioactive compound in the extract. With that reference, the GC–MS results of the grape seed extract revealed that the bioactive compound 1, 3-benzenediol (35.8%) has the highest peak area percentage, followed by 1,3,5-benzenetriol (33.3%), 4-vinylbenzene-1, 2-diol (16.3%), eicosanoic acid (3.3%), benzoic acid (2.8%), reticuline (2.6%), 2-hydroxy-5-methylisophthalaldehyde (2.0%), cyclohexanecarboxylic acid (1.7%), octadecanoic acid (1.4%), ad 2,6,10,14,18-pentamethy l - 2, 6, 10, 14, 18-eicosapentaene (1.0%) **(Figure 1 and Table 1).** The antioxidant activity of the grape seed methanolic extract (IC_{50}) was 212.6 µg/kg by the DPPH method.

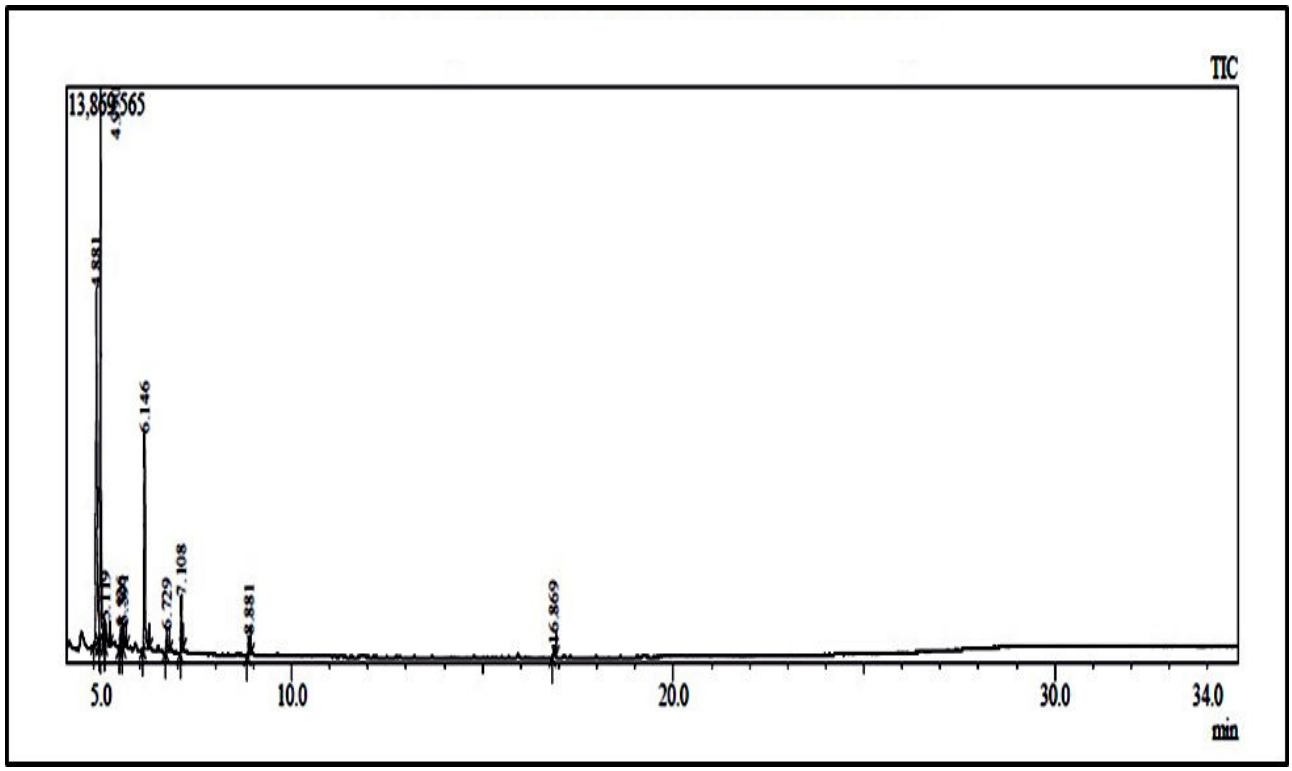

Figure 1. GC–MS analysis of grape seed methanol extract with their chemical groups.

Table 1. GC–MS analysis of grape seed methanol extract with their chemical groups.

Peak #	Retention time	Area	Area (%)	Height	Height (%)	A/H	Name of the compound	Chemical groups
1	4.881	19378113	33.1	8552305	27.3	2.3	1, 3, 5-Benzenetriol	Polyphenol
2	4.990	20911678	35.8	13440619	43.0	1.6	1, 3-Benzenediol	Phenol
3	5.119	165980	2.8	539696	1.7	3.1	Benzoic acid	Phenolic acid
4	5.526	1538221	2.6	48317	1.6	3.2	Reticuline	Alkaloid
5	5.591	1153355	2.0	529976	1.7	2.2	2-Hydroxy-5-methylisophthalaldehyde	Aldehyde
6	6.146	953739	16.3	5187019	16.6	1.8	4-Vinylbenzene-1, 2-diol	Phenol
7	6.729	993937	1.7	524958	1.7	1.9	Cyclohexane carboxylic acid	Aromatic carboxylic acid
8	7.108	1932740	3.3	1330033	4.3	1.5	Eicosanoic acid	Straight-chain saturated fatty acid
9	8.881	813359	1.4	32017	1.4	1.8	Octadecanoic acid	Straight-chain saturated fatty acid
10	16.869	588783	1.0	26120	0.8	2.3	2, 6, 10, 14, 18-Pentamethyl - 2, 6, 10, 14, 18-eicosapentaene	NK
		58499905	100.0	31281860	100.0			

Discussion

V. vinifera is one of the highly consumed fruits globally and widely used in the wine industry. Grape seeds are considered industrial wastes or byproducts of wine preparation. Nevertheless, grape seeds are a rich source of polyphenols. Nearly 60.0%–70.0% of the total extractable phenolic compounds (catechin, epicatechin, procyanidins, and their polymers) are present in the seeds. Various external factors including the cultivar, climatic condition, geographic origin, and degree of maturation determine the phenolic contents of the grapes. Few studies have reported that grape seed extract is commercially available as a dietary supplement approved by everything added to food in the United States, Food and Drug Administration, and is generally recognized as safe. [23] In this study, the phytocompounds present in the methanolic extract of grape seeds were identified by GC–MS. The phytochemical profiling of the methanolic extract revealed the high contents of phenols (1, 3-benzenediol, 4-vinylbenzene-1, 2-diol), followed by polyphenol (1, 3, 5-benzenetriol), straight-chain saturated fatty acid (eicosanoic acid and octadecanoic acid), phenolic acid (benzoic acid), alkaloid (reticuline), aldehyde (2-hydroxy-5-methylisophthalaldehyde), and carboxylic acid (cyclohexane carboxylic acid). The antioxidant activity of the grape seed extract was 212.6 µg/kg. Antioxidant studies reported that the grape seed extract showed higher antioxidant activity than standard antioxidants (vitamins E and C). [24] Rababah TM, *et al*. reported antioxidant activities of the extracts of different cultivars of grape seeds; however, no significant changes in the total phenolic contents were found. [25] Based on previous studies, the phytocompounds benzoic acid and reticuline could serve as sources of potential natural antioxidants. [26, 27]

The antioxidant potential of phenolic compounds could be linked to the free radical scavenging capacity (direct) and enhancing the activity of antioxidant enzymes (indirect). Thus, plant phenolic compounds are increasingly explored for their preventive roles in chronic and oxidative stress-related disorders such as cancer, cardiovascular diseases, and neurodegenerative diseases. In this study, the phytochemical profiling of grape seed extract revealed various phenolic compounds including phenols (1, 3-benzenediol, 4-vinylbenzene-1, 2-diol), polyphenol (1, 3, 5-benzenetriol), phenolic acid (benzoic acid), and alkaloid (reticuline). Grape seed procyanidin oligomers were reported to be highly potent antioxidants than vitamin C (20 times more) and (50 times more). [28] Grape seed proanthocyanidins possess both hydroxyl and superoxide radical scavenging activity. [29] Animal studies have documented that grape seed proanthocyanidins exhibit superior antioxidant activity (lipid peroxidation and DNA fragmentation) compared with vitamin E, vitamin C, vitamins E + C, and beta-carotene. [30] The methanolic extract of grape seeds was found to possess an antioxidant capacity of 212.6 µg/kg. Of the phytocompounds identified in the grape seed extract, benzoic acid and reticuline are potent natural antioxidants.

This study had evaluated the antioxidant potential of the crude methanolic extract of grape seeds. However, further studies are warranted to assess the antioxidant potential of the individual phytocompounds, particularly reticuline and benzoic acid in grape seeds.

Conclusion

The findings of this study indicate that grape seed extract is a potent source of natural antioxidants that can be used as a safe alternative to improve the quality and shelf-life of food compared with other synthetic chemical antioxidants. Therefore, the grape seed extract can be used as a promising antioxidant/free radical scavenging candidate with wider therapeutic applications in metabolic disorders, inflammatory conditions, and cancer.

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Conflicts of interest statement

All the authors have completed the ICMJE disclosure form for potential conflicts of interest. All the authors confirm that there is no conflict of interest to disclose.

Data sharing statement

Data sharing statement. All data generated or analyzed during the present study are included in this published article. Further details are available for noncommercial purposes from the corresponding author on reasonable request.

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